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The olive cultivar 'Picual' is an optimal pollen donor for 'Barnea'

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ABSTRACT

Some olive (*Olea europaea* L.) cultivars are almost completely self-incompatible. The aim of this study was to determine the optimal pollen donors for the olive cultivar 'Barnea'. The study was carried out during two consecutive years and was based on artificial cross-pollination as well as molecular paternity analysis of 'Barnea' fruits sampled from commercial olive orchards. We assessed the dates and duration of the flowering of 11 commercial olive cultivars to determine each cultivar's optimal period of effective cross-pollination. Artificial cross-pollination of 'Barnea' flowers with the donors 'Picual', 'Coratina' and 'Askal' showed fruit set rates similar to those obtained in open-pollination. 'Arbequina' showed a much lower fruit set rate and self-pollination of 'Barnea' proved it to be close to self-incompatible under our conditions. The stigma was found to be receptive to pollen during the first four days after anthesis. Self pollination resulted in germination of the pollen grains on the stigma. However, the pollen tubes were significantly shorter than the ones resulting from cross-pollination. Based on molecular paternity analysis, only three seeds out of 202 analyzed 'Barnea' fruits, sampled from commercial orchards, were found to be the product of self-pollination. 'Picual' was identified as the most frequent pollinator of 'Barnea' trees in commercial olive orchards, even when located further away from the 'Barnea' trees than various other cultivars.

The cv. 'Picual' has been tentatively identified as a superior pollinator of the cv. 'Barnea' based on several parameters, leading us to suggest that the yield of 'Barnea' may improve if there are 'Picual' trees present upwind to their location in the olive plantation.

1. Introduction

Since antiquity, olive oil and table olives have been major components of the daily diet and culture of the various ethnic groups of the Middle East. Commercial olive orchards in Israel consist of 1800 ha devoted to production of table olives and 29,000 ha of trees for oil production. One quarter of Israel's oil producing trees grow in intensively-managed, irrigated orchards, planted at a density of 4×6 m and irrigated with an average of 500 mm drip irrigation per year in addition to the annual rainfall. The 'Barnea' cultivar makes up half of these trees. The viability of the olive industry depends on harvesting a profitable yield of fruit, whether used for oil production or as table olives. Obviously, in table olives, fruit quality and appearance are important parameters as well. Despite the abundance of flowers on olive trees during the flowering season, only a few set fruit during the growing season and of these, only about 1–5% reach maturity as fully developed fruits (El-Hady et al., 2007; Pinillos and Cuevas, 2009; Wu et al., 2002). Several factors are responsible for this low fruit to flower ratio including the genetic background of the specific cultivar, climatic conditions during fruit set, compatibility relationships between parent cultivars and other parameters. In some cases, this ratio may be the result of a high proportion of male flowers (Ayerza and Coates, 2004; Koubouris et al., 2009; Lavee et al., 2002). Generally however, the number of male flowers does not affect the level of the yield (Lavee, 1996).

Some cultivars are nearly self-incompatible, which means that the flowers are not fertilized by pollen of the same cultivar. Other cultivars are cross-incompatible, in which case flowers cannot or nearly not be fertilized by pollen from certain other cultivars. The degree of self-incompatibility varies among cultivars (Cuevas and Polito, 1997; Diaz et al., 2006; El-Hady et al., 2007; Lavee et al., 2002; Moutier, 2002; Wu et al., 2002).

Self-incompatibility is governed by one locus (S-Locus) that directs two different mechanisms in plants: gametophytic self incompatibility (GSI) and sporophytic self incompatibility (SSI). The olive self incompatibility mechanism is unknown and during

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the last several years, some studies supported the GSI model (Ateyyeh et al., 2000; Cuevas and Polito, 1997; Serrano and Olmedilla, 2012; Wu et al., 2002), whereas others supported the SSI mechanism (Breton and Berville, 2012; Collani et al., 2010).

It is important for growers to take into account the degree of cross-compatibility between cultivars when planning an olive orchard in order to maximize fruit set and yield. This is especially important when orchards are planted in isolated areas where the only sources of pollen available are within the orchard. However, olive pollen grains can traverse distances of more than 20–30 km, and olive pollen is abundant both in 'on' and 'off' years (Ferrara et al., 2007; Mazzeo et al., 2014). Since the pollination process may vary from year to year and can be affected by environmental factors, studying pollen flow among different cultivars at the molecular level is a relatively new and exciting approach that may prove to be a more reliable and powerful method of determining compatibility than studies based on artificial hand pollination, bagging, pollen tube growth, or fruit set. The identification of suitable pollen donors uses paternity analysis to examine the paternal genetic contribution in the offspring and thus identify the most likely male genitor. Olive flowers bear large quantities of pollen and are wind pollinated (Cuevas and Polito, 2004). Lavee et al. (2002) studied the significance of cross-pollination for various olive cultivars under intensive growing conditions. They recommended a specific pollinator for each of the various cultivars. Lavee's team enclosed the flowers in bags, and at the time of anthesis, added flowering branches of the pollinator cultivar. The number of fruits was counted two months after bloom in each case and in comparable controls. However, when flowers are bagged, the microenvironment differs from the natural conditions under which fruit set usually takes place. In addition, some pollen of other cultivars might be present on the leaves and could be a possible source of contamination with unintended cross-pollination. Because of the high rate of fruit loss in olives, the results of such studies depend significantly on whether initial or final yield is considered (Mookerjee et al., 2005).

De la Rosa et al. (2004) used paternity analysis with data from four microsatellite markers to verify the parents of seedlings thought to be derived from self-pollination or controlled crossing. They found that a high level of contaminating pollen had breached the pollination bags during manual pollination. Diaz et al. (2006) also found contamination in pollination bags. These findings throw many previous results using pollination bags into question, especially if the bags were not placed over the inflorescences well before anthesis. In contrast, compatibility relationships based on paternity analysis can reliably trace genes in mature fruits back to the mother tree and its pollinator, so that the true pollen donor can be identified. Mookerjee et al. (2005) used eight microsatellite markers to identify 17 genotypes that were potential pollen donors in a commercial olive orchard. DNA typing with the same primers was then applied to 800 olive embryos collected from five cultivars and pollen donors were assessed by paternity analysis, based on the paternal contribution of alleles to the genotypes of the embryos. This study has shown that the ability to trace the contribution of the male parent to the genome of the offspring by molecular techniques is a reliable tool in assessing the mutual compatibility of olive cultivars. Based on paternity analysis, 'Kalamata' was found to be highly self-incompatible whereas 'Barnea', 'Benito', and 'Katsourela' were found to be efficient pollen donors to 'Kalamata' (Seifi et al., 2012).

The aim of the current study was to determine the optimal pollen donors for the 'Barnea' cultivar, which is one of the major cultivars in the modern intensive large scale olive orchards, using artificial cross-pollination and paternity analysis.

2. Materials and methods

2.1. Characterization of the flowering period

2.1.1. The current study was performed during 2011 and 2012

Characterization of the flowering time for each cultivar was performed in a 15 year old irrigated olive orchard of the Agriculture Research Organization (ARO) at Bet Dagan, Israel. This orchard consists of three trees from each of 150 olive cultivars spaced 5×6.5 m apart. Six external branches with about 100 flower buds, from each tested cultivar, were marked and the actual number of buds on each branch was recorded. The trees were observed at regular intervals of 3–4 days from the beginning of March until the end of May. At each observation, the number of closed buds, open flowers and dried flowers was recorded.

2.2. Artificial cross-pollination

Cross-pollination was performed in a 9 year old irrigated 'Barnea' olive orchard at Benei Darom in the southern coastal plain of Israel. Artificial cross-pollination was carried out by hand. For each donor tested, 15 'Barnea' branches from 15 trees, each containing 50 flowers at the white stage before opening (stage 3 at Fig. 1a), were marked and all other flowers, which were not at the white stage, were removed. The stamens of the 50 buds were removed and flowers were then pollinated by brushing the pistils with the donor pollen (Fig. 1b). All flowers were then enclosed in a paper bag and after two months, the number of normal developing fruits was determined.

Prior to artificial pollination, pollen viability was tested by in vitro germination experiment on a sucrose media (10% sucrose and 0.8% agar).

2.3. Stigma receptivity analysis

Stigma receptivity was assessed after hand pollination of 'Barnea' flowers with pollen extracted from 'Barnea' and 'Askal' flowers. Five 'Barnea' 5 years old trees, in 10 l pots, were placed, at the beginning of February (for two months), in a closed growth chamber set at $20 \pm 2\,^{\circ}$C and 60% humidity, under 15 h of illumination per day. During four consecutive days within the flowering period, the opening date of each flower was recorded. In the fourth day, 10 'Barnea' flowers from each opening date were pollinated by brushing the stigma with pollen from one of the pollen donors–'Askal' or 'Barnea'. Pollen viability was tested before pollination as mentioned.

The flowers were harvested 24 h after pollination and preserved in 70% ethanol at $4\,^{\circ}$C for later study of pollen germinability on the stigma according to the aniline blue epifluorescence method (Martin, 1959). The numbers of pollen grains and pollen tubes as well as tube length on the stigma were recorded with an Olympus IX 81 fully automated laser scanning confocal microscope (Olympus, Tokyo, Japan).

2.4. Paternity analysis

For molecular paternity analysis, one fruit was collected from all four sides of five 'Barnea' trees in each of eight commercial olive orchard distributed all over Israel (Supplementary Table 1). In 2012, two plots were added (Magal 1 and 2). All commercial cultivars grown in Israel were characterized based on 16 SSR markers (Biton et al., 2012) and extracted embryo DNA samples were genotyped using the five most polymorphic SSRs for the identification of the pollen donor cultivar.

For each analyzed tree, the closest cultivar in each of the four directions was designated as a neighbor. Then, the identity of the